

A COMPARATIVE STUDY OF BACILLUS
PULLORUM AND BACILLUS
SANGUINARIUM

A DISSERTATION
SUBMITTED TO THE FACULTY
OF THE OGDEN GRADUATE SCHOOL OF SCIENCE
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF HYGIENE AND BACTERIOLOGY

BY
FREDERICK WILLIAM MULSOW

Private Edition, Distributed By
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE JOURNAL OF INFECTIOUS DISEASES, Vol. XXV, No. 2
August 1919



Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41546816_0011

The University of Chicago

A COMPARATIVE STUDY OF BACILLUS
PULLORUM AND BACILLUS
SANGUINARIUM

A DISSERTATION

SUBMITTED TO THE FACULTY
OF THE OGDEN GRADUATE SCHOOL OF SCIENCE
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF HYGIENE AND BACTERIOLOGY



BY

FREDERICK WILLIAM MULSOW

• Private Edition, Distributed By
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE JOURNAL OF INFECTIOUS DISEASES, Vol. XXV, No. 2
August 1919

THE DIFFERENTIATION AND DISTRIBUTION OF THE PARATYPHOID-ENTERITIDIS GROUP. VI

AVIAN PARATYPHOID BACILLI: A COMPARATIVE STUDY OF *B. PULLORUM* AND *B. SANGUINARIUM*

F. W. MULSOW

From the Department of Hygiene and Bacteriology, University of Chicago

Two organisms of the paratyphoid-enteritidis group designated, respectively, as *B. pullorum* and *B. sanguinarium*, are considered responsible for certain diseases of the domestic fowl. They are generally regarded as quite widely distributed thruout the United States. We have, nevertheless, little definite knowledge at present as to the prevalence of the diseases caused by these organisms. Smith and Ten Broeck¹ have suggested that *B. sanguinarium* may be the cause of the occasional reports of gastro-intestinal disturbances following the eating of meat of fowls. The feeding experiments of Rettger, Hull and Sturges² suggest that *B. pullorum* may be the cause of food poisoning. The disease in fowls produced by *B. sanguinarium* resembles clinically that caused by *B. avisepticus*, and Hadley³ states that many of the laboratory cultures of *B. avisepticus* are really *B. gallinarum* or some member of the paratyphoid-enteritidis group. Several investigators have observed the close relationship of these organisms to each other, their antigenic and cultural relations to *B. typhosus*, and the general relationship of these organisms to the paratyphoid-enteritidis group. In view of these facts it appears that further study of these organisms is quite desirable.

B. sanguinarium was first isolated in the United States in 1894 by Smith,¹ from an epidemic among fowls in Rhode Island. Moore⁴ isolated the same organism in 1895 from diseased fowls in Washington, D. C., Virginia, and Maryland and gave the first detailed description of the organism and the disease produced by it. He at first called the disease "infectious leukemia" because of the reduction in number of the red blood cells and the increase in the number of white blood cells. Curtis⁵ studied an outbreak of the disease in Rhode Island in 1901 and called the disease "fowl typhoid," which is the name accepted today. *B. sanguinarium* has been isolated more recently

Received for publication March 13, 1919.

¹ Jour. Med. Research, 1915, 31, p. 503.

² Jour. Exper. Med., 1916, 23, p. 475.

³ Jour. Bacteriol., 1918, 3, p. 286.

⁴ Twelfth and Thirteenth Annual Reports, Bur. An. Ind., U. S. Dept. of Agric., p. 185.

⁵ R. I. Exper. Sta., Bull. 87, 1902.

from several epidemics in the New England states. Pfeiler and Rehse⁶ in 1913 described what was apparently the same organism as the cause of an epidemic among fowls in Germany. They named it *B. typhi gallinarum* alcalifaciens, apparently not knowing that it had been previously described by Moore and others. Pfeiler and Roepke⁷ have isolated this organism from a second epidemic in Germany and have given quite a thoro description of it, which shows that it is undoubtedly the same organism described by Moore. Lignières and Zabala⁸ isolated a paratyphoid-like organism from fowls during an epidemic among fowls in South America and from their description, the bacterium appears to be very similar if not identical to *B. sanguinarum*. It also appears that Klein,⁹ as has been suggested by Hadley in a personal communication, described the same organism in 1889 under the name *B. gallinarum* as the cause of an epidemic in England. Altho his description is not as complete as has been given in recent years by Moore and others, yet his description and my own work with this organism indicate that it is identical with the one described by Moore. Since the organism studied by Klein apparently has the priority in nomenclature, it appears that the name of the bacillus of fowl typhoid should be *B. gallinarum*. Final decision on this point, however, should await a classificatory revision of the whole group. In order to avoid confusion at this time I shall use the name *B. sanguinarum*.

B. pullorum as the cause of the disease known as "bacillary white diarrhea" was first isolated in 1899 by Rettger¹⁰ from young chicks which had died of the disease. There has been some doubt, at times, as to whether this organism was really the cause of white diarrhea of chicks. Morse¹¹ in 1909 observed that this disease was due to a coccidium. A recent Farmers' Bulletin (957) issued by the Bureau of Animal Industry, Washington, D. C., states that a clinical disease called "white diarrhea" of chicks may be caused by *B. pullorum*, *B. avisepticus*, a coccidium, or *Aspergillus fumigatus*. During the past few years *B. pullorum* has been the cause of epidemics among chicks in many parts of the United States. It has been observed, however, much more frequently in the New England states. Young chicks under 72 hours of age are considered to be especially susceptible, altho Jones¹² and Hadley¹³ have described epidemics among adult fowls, due to this organism.

The pathologic changes produced by *B. pullorum* have been thoroly studied by Gage and Martin.¹⁴ They state that the disease in small chicks is a septicemia and that the minute necrotic foci in the liver, spleen, and pancreas are the most characteristic lesions. If the chick recovers from an infection it may become a permanent carrier. Horton¹⁵ reports the apparent recovery of a bantam hen which evidently had an ovarian infection, since two of her eggs contained the organism and the agglutination test with her serum was positive; however, a year later the organisms could not be isolated from the eggs and the agglutination reaction was negative. The ovary seems to be the most

⁶ Mitth. d. Kaiser-Wilhelms Inst. f. Landwirtschaft in Bromberg, 1913, No. 4, p. 306.

⁷ Centralbl. f. Bakteriologie, 1917, 79, p. 125. (Abstracted in Jour. Comp. Path. and Therap., 1917, 30, p. 263.)

⁸ Hoare, A System of Veterinary Med., 1913, 1, p. 466.

⁹ Centralbl. f. Bakteriologie, 1889, 5, p. 689.

¹⁰ N. Y. Med. Jour., 1900, 71, p. 803.

¹¹ U. S. Dept. of Agric., B. A. I. Circular 128 (1909).

¹² Jour. Med. Research, 1913, 27, p. 471.

¹³ R. I. Exper. Sta., Bull. 172.

¹⁴ Jour. Med. Research, 1916, 34, p. 149.

¹⁵ Jour. Bacteriology, 1916, I, p. 625.

frequent site of the infection in adult hens and often has a pathologic appearance. However, apparently normal ova may carry the organism to the full formed egg.

In the present study of *B. pullorum* and *B. sanguinarium* comparisons have been made with typical strains of members of the paratyphoid-enteritidis group, and with *B. typhosus* and *B. avisepticus*. Typical representatives of the paratyphoid-enteritidis group were obtained from Jordan¹⁶ who has made an extensive study of this group. For the history of these strains the reader may refer to his publications. Typical strains of *B. typhosus* were taken from stock cultures. It was somewhat more difficult to secure typical strains of *B. avisepticus*, hence it was necessary to devote some time to the study of this organism. Altho an organism was described under this name as early as 1879 by Toussaint and in 1880 by Pasteur, yet there are very few data concerning its fermentative action on the carbohydrates and, furthermore, there is considerable disagreement as to many of its characteristics, such as fermentative action, indol production, and pathogenesis. It is generally agreed, however, that this organism is a short gram-negative, nonmotile rod which does not liquefy gelatin. It is usually described as having a marked tendency to stain deeper at the poles, especially when stained from the blood of an infected fowl. The tendency to polar staining, however, is markedly reduced when grown on artificial mediums or when inoculated into rabbits; hence it is often quite difficult to distinguish it from *B. sanguinarium*, which frequently stains lightly in the central part. *B. avisepticus* is usually highly virulent for rabbits, but may soon lose this virulence when grown on artificial mediums. This may explain in part the contradictory results observed by various investigators concerning its pathogenesis. In view of these facts it seemed advisable to include the reaction of strains of *B. avisepticus* in the tables and in certain discussions.

The organisms which have been studied in the present work were obtained from as widely different sources as possible and were tested for purity by plating on Endo medium. Three colonies were picked from the Endo plates and compared with the original, in milk containing brom-cresol-purple as an indicator and in dextrose, lactose and maltose broth fermentation tubes containing an indicator to show any change in the acidity as well as gas production. The source of the cultures is given in table 1.

¹⁶ Jour. Infect. Dis., 1917, 20, p. 457.

TABLE - 1
SOURCE OF CULTURE

Strain	Original Label	Received from	Isolation		
			Date	Location	By whom
B. sanguinarium					
1	B. of Fowl typhoid I	Dr. Theobald Smith...	1895	Va. or Md.	Dr. V. A. Moore
2	B. of Fowl typhoid II	Dr. Theobald Smith...	1894	R. I.	Dr. Theobald Smith
3	B. sanguinarium 781	Am. Mus. Nat. Hist. ...	Mar. '17	N. Y.	Dr. Chas. Krumweide
11	B. sanguinarium "Taylor"	Dr. W. A. Hagan.....	Apr. '14	Calif.	Dr. W. J. Taylor
12	B. sanguinarium "Smith IV"	Dr. W. A. Hagan.....			Dr. Theobald Smith
13	B. gallinarum No. 51 or Rettgers cult. "S"	Dr. P. B. Hadley.....		Conn.	Dr. L. F. Rettger
14	B. gallinarum No. 108	Dr. P. B. Hadley.....	Oct. '14	R. I.	Dr. P. B. Hadley
15	B. gallinarum No. 110	Dr. P. B. Hadley.....	Oct. '14	R. I.	Dr. P. B. Hadley
50	B. gallinarum No. 42	Dr. P. B. Hadley.....		R. I.	Dr. P. B. Hadley
51	B. gallinarum No. 45	Dr. P. B. Hadley.....		R. I.	Dr. P. B. Hadley
52	B. gallinarum No. 66	Dr. P. B. Hadley.....		Conn.	Dr. P. B. Hadley
53	B. gallinarum No. 69	Dr. P. B. Hadley.....		N. Y.	
54	B. gallinarum No. 115	Dr. P. B. Hadley.....	1892		Dr. V. A. Moore
88	B. gallinarum No. 88	Dr. P. B. Hadley.....	1888	Kent, Eng.	Dr. E. Klein
55	From cholera-like epidemic	Dr. Chas. Murray.....	1917	Iowa	Dr. Chas. Murray
47	B. sanguinarium		Apr. '18	Ill.	F. W. Mulsow
56	From cholera-like epidemic	Dr. Chas. Murray.....	Oct. '18	Iowa	Dr. Chas. Murray
57	B. sanguinarium		Nov. '18	Ill.	F. W. Mulsow
40	B. avisepticus No. 549	Am. Mus. Nat. Hist. ...	1908	Colo.	State Vet. Coll.
41	From cholera-like epidemic among wild ducks	Univ. of Kansas.....	1916	Kans.	Dr. B. J. Clawson
42	B. avisepticus "Park"	Univ. of Kansas.....			Dr. W. H. Park
B. avisepticus					
44	B. avisepticus	B. A. I., Wash., D. C.		R. I.	Dr. P. B. Hadley
45	B. avisepticus	L. M. Roderick.....	Feb. '18	N. D.	L. M. Roderick
46	B. avisepticus	L. M. Roderick.....	Mar. '18	N. D.	L. M. Roderick
48	B. avisepticus No. 62 (Kral No. 3)	Dr. P. B. Hadley.....			Kral's laboratory
49	B. avisepticus No. 91	Dr. P. B. Hadley.....		R. I.	Dr. P. B. Hadley
71	B. avisepticus		Nov. '18	Ill.	F. W. Mulsow
72	B. avisepticus		Oct. '18	Ill.	F. W. Mulsow
B. pullorum					
25	B. gallinarum No. 102A	Dr. P. B. Hadley.....		R. I.	Dr. P. B. Hadley
4	B. pullorum IV	Dr. Theobald Smith...			Dr. Theobald Smith
5	B. pullorum No. 5	Dr. C. M. Haring.....	July '14	Calif.	Dr. C. M. Haring
6	B. pullorum VI	Dr. Theobald Smith...			Dr. Theobald Smith
7	B. pullorum	Dr. G. D. Horton.....	1917	Mo.	Dr. G. D. Horton
8	B. pullorum	Dr. G. D. Horton.....	1917	Mo.	Dr. G. D. Horton
9	B. pullorum No. 9	Dr. C. M. Haring.....	Jan. '17	Calif.	Dr. C. M. Haring
10	B. pullorum	Dr. L. F. Rettger.....	1909	Conn.	Dr. L. F. Rettger
16	B. pullorum	Dr. L. F. Rettger.....	1916	Conn.	Dr. L. F. Rettger
18	B. pullorum "K Rettgers"	Dr. W. A. Hagan.....			
19	B. pullorum J2	Dr. W. A. Hagan.....		N. Y.	Dr. F. S. Jones
20	B. pullorum	Dr. W. A. Hagan.....		N. Y.	Dr. F. S. Jones
21	B. pullorum 17	Dr. P. B. Hadley.....		R. I.	Dr. P. B. Hadley
22	B. pullorum 34	Dr. P. B. Hadley.....		R. I.	Dr. P. B. Hadley
23	B. pullorum 56	Dr. P. B. Hadley.....		R. I.	Dr. P. B. Hadley
24	B. pullorum 99	Dr. P. B. Hadley.....		R. I.	Dr. P. B. Hadley
26	B. pullorum F4	Dr. L. F. Rettger.....	1910	Conn.	Dr. L. F. Rettger
27	B. pullorum (W. R.)	Dr. L. F. Rettger.....	1911	Conn.	Dr. L. F. Rettger
28	B. pullorum B	Dr. L. F. Rettger.....	1916	Conn.	Dr. L. F. Rettger
29	B. pullorum W	Dr. L. F. Rettger.....	1917	Conn.	Dr. L. F. Rettger
30	B. pullorum 403	Am. Mus. Nat. Hist. ...	1911	N. Y.	Dr. F. S. Jones
31	B. pullorum	Dr. F. D. Beckwith....	Jan. '18	Oreg.	Dr. F. D. Beckwith
32	B. pullorum	B. A. I., Wash., D. C.		D. C.	

DISTRIBUTION

In order to learn more concerning the distribution of these organisms and to secure cultures from as widely different sources as possible, letters were written to every agricultural experiment station in the United States. I wish to express my thanks at this time for cultures and information received from the many correspondents.

From publications and letters from the experiment stations it appears that investigations concerning these organisms have been carried on in only a few states. Hence our knowledge of the diseases caused by these organisms in the United States is quite fragmentary and rests on scattered observations. A few states as Connecticut, Rhode Island and Massachusetts have made quite thoro studies of these organisms and methods of controlling their spread. Their distribution, as obtained from the correspondence and bulletins of the several experiment stations is summarized in table 2.

TABLE 2
DISTRIBUTION OF *B. PULLORUM* AND *B. SANGUINARIUM* BY STATES

No Epidemics Observed	No Studies Made	Occurrence of <i>B. pullorum</i>		Occurrence of <i>B. sanguinarium</i>	
		Rare	Frequent	Rare	Frequent
Ala. Colo. Mich. Minn. Miss. N. J. N. C. *N. D. *Ohio Okla. *S. D. Vt. W. Va.	Ariz. Fla. Idaho Ky. Maine Md. Mont. Nev. N. Mex. S. C. Utah Va.	*Iowa Kans. Nebr. Texas Wisc. La.	Calif. Conn. *Del. *Ill. Ind. Mass. Mo. N. H. N. Y. Ore. Penn. R. I.	Calif. Del. Iowa Mo. Penn. Wash. Wyo.	Conn. Mass R. I.

* Letters from these states contain the information that chicken cholera is rather prevalent.

From table 1 it appears that *B. sanguinarium* has been isolated comparatively few times, but as has been suggested by Taylor¹⁷ and Hadley³ and from my own observations, it appears that this organism is more frequently the cause of epidemics in fowls than it is generally considered to be. Thus, some cultures which I have received from other laboratories that were labelled *B. avisepticus* have proven to be *B. sanguinarium*. Hence many cholera-like epidemics attributed to *B. avisepticus* may very likely be due to *B. sanguinarium*.

¹⁷ Jour. Am. Vet. Med. Assn., 1916, 49, p. 35.

CULTURAL CHARACTERISTICS

Quite thoro descriptions of the staining, morphological, and cultural characteristics on the ordinary mediums of *B. sanguinarium* and *B. pullorum* have been made by Moore⁴ and Rettger,¹⁸ respectively. It seems necessary to mention only a few of their most characteristic reactions. They are gram-negative, nonmotile rods, and belong to the paratyphoid-enteritidis group. *B. sanguinarium* does not, in general, take the ordinary stains as readily as *B. pullorum*, and often when stained with fuchsin the central portion is only faintly stained. The growth on agar and gelatin resembles that of the other members of the paratyphoid-enteritidis group, altho in my work the strains of *B. pullorum* have not grown as a rule as luxuriant on these mediums as *B. sanguinarium* or other members of this group.

INDOL

In Moore's original article it is stated that *B. sanguinarium* produces a slight amount of indol, but other investigators using the more accurate modern methods have not been able to detect indol formation by this organism. *B. pullorum* has never been observed to produce indol.

The test for indol production in the present study has been made in two ways, on three different occasions. The first time the organisms were grown in the standard peptone solution for 1-5 days, respectively. In the second and third tests the organisms were grown in standard meat extract broth and in meat infusion broth, respectively. The test for indol has been made in two ways, with the same results in all cases.

1. By Ehrlich's paradimethyl-amido-benzaldehyde reaction.

2. By the use of vanillin as described by Nelson (J. Biol. Chem., 1916, 24, p. 527).

Control tests were made by testing uninoculated broth, and cultures of *B. Coli*.

Indol was not produced by any strains of *B. pullorum* or *B. sanguinarium*. Seven strains of *B. avisepticus* produced indol, but two strains, 40 and 42, which were labelled *B. avisepticus* did not form indol. These two strains have proven to be similar to strains of *B. sanguinarium* in many other respects; hence, I have called them *B. sanguinarium*. There is some dispute as to the ability of *B. avisepticus* to produce indol. Lignières¹⁹ states that indol is not produced by this organism, while Kitt¹⁹ and others maintain that indol is pro-

¹⁸ Jour. Med. Research, 1908, 18, p. 227.

¹⁹ Handb. d. pathogen, Mikroorganismen, 1913, 6, p. 39.

duced by *B. avisepticus*. My results have been in accord with those of Kitt.

HYDROGEN SULPHID

Since it has been observed by Burnet and Weissenbach,²¹ Jordan and Victorson,²² and others, that certain members of the paratyphoid-enteritidis group differ in their ability to produce hydrogen sulphid, it seemed desirable to test the avian strains in this respect.

The test for hydrogen sulphid production was made by placing 2 drops of a 10% aqueous solution of lead acetate into tubes containing 10 cc of sterile freshly prepared nutrient agar cooled to about 50 C. The tubes were then cooled and stabs were made by passing the inoculating needle between the wall of the tube and the agar. Tests have been made at three different times. The first time meat extract was used in the preparation of the agar and only the strains from 1-20 were tested. In the second, meat infusion broth was used in the preparation of the agar. The third was made according to the method of Jordan and Victorson,²² with the exception that my medium contained Armour's peptone instead of Witte's.

All the strains of *B. pullorum* and *B. sanguinarium*, except *B. pullorum* No. 19 and *B. sanguinarium* No. 3 and No. 14 produced a blackening of the medium along the line of inoculation in 24 hours when tested by the method of Jordan and Victorson. In the second test the *B. sanguinarium* No. 14 was positive in 24 hours and *B. sanguinarium* No. 54 was positive only after 48 hours. These strains which do not form hydrogen sulphid in 24 hours are typical in other respects. On the whole, these avian strains are like *B. enteritidis* and *B. paratyphoid B* in regular hydrogen sulphid production.

MILK

It has been stated by Smith and Ten Broeck,¹ Rettger and Koser,²³ and others that *B. sanguinarium* produces an alkaline reaction in milk in a few days, while *B. pullorum* remains acid. Their observations extended over a period of about two weeks.

In the present study I used certified milk from which the cream had been removed. The milk was sterilized by heating in the autoclave for 15-20 minutes at 10 lbs. of pressure. Either 7% of a 1% solution of Merck's litmus or 1% of a 0.5% solution of brom-cresol-purple prepared as recommended by Clark and Lubs, was added to the milk before sterilization. The two indicators have given practically the same results altho the brom-cresol-purple appears to be the more delicate and is not so easily reduced.

²¹ Compt. rend. Soc. de biol., 1915, 78, p. 565.

²² Jour. Infect. Dis., 1917, 21, p. 571.

²³ Jour. Med. Research, 1917, 35, p. 443.

All strains produce a slight acidity in 24-48 hours. The strains of *B. sanguinarium* from alkali, however, after 2-5 days. There are some exceptions to this which are shown in table 3. All strains of *B. pullorum* produce alkali after 60-days' incubation. Some strains showed an alkaline reaction as early as 4 days, while others did not become alkaline until about 60 days. The reaction varies considerably with different lots of milk.

Since certain strains of *B. pullorum* form alkali as early as some strains of *B. sanguinarium* it appears that we cannot differentiate between these two organisms by any change produced in the reaction of milk. Altho, in general, *B. sanguinarium* produces alkali earlier than *B. pullorum*. The *B. avisepticus* produces a slight acidity in 48 hours which persists for 90 days, but the organisms are usually dead after about 2 weeks' incubation. Thus it may be distinguished from the other organisms by its action on milk.

The digestive action of these organisms on the casein was tested with milk which contained no indicator. *B. sanguinarium*, in general, completely digested the casein in about 2 weeks, altho with some strains digestion was not complete before 6 weeks. The strains of *B. pullorum* vary even more in their digestive action; with some strains digestion was complete in 2 weeks, while others have failed in this respect in 90 days. Therefore these organisms cannot be differentiated by their digestive action on milk.

Details of the changes in the reaction of milk produced by these organisms are given in table 3.

FERMENTATION OF CARBOHYDRATES

The fermentation reactions of *B. sanguinarium* and *B. pullorum* have been studied quite extensively by Rettger and Koser,²³ and more recently by Krumwiede and Kohn.²⁴ The former investigators state that the principal fermentative differences between these organisms are: (1) The production of gas by *B. pullorum* in dextrose, levulose, galactose, and mannite, and the inability of *B. sanguinarium* to form gas in any of the carbohydrates, and (2) the production of acid in maltose, dulcitol, and dextrin by *B. sanguinarium* and the failure of *B. pullorum* to ferment these substances. Similar reactions have been observed by Goldberg²⁵ and others.

The ability of *B. pullorum* to form gas in some carbohydrates seems to vary with different strains and at times the same strain will show variations in its ability to produce gas. Rettger isolated a strain which did not form gas in any of the carbohydrates. He has also observed variations in gas pro-

²⁴ Ibid., 1917, 36, p. 509.

²⁵ Jour. Am. Vet. Med. Assn., 1917, 51, p. 203.

duction with other strains. Variation in the ability to form gas has also been noted by Krumwiede and Kohn,²⁴ who secured a non-gas producing strain from Smith, but in their work this strain was observed to produce gas.

TABLE 3
CHANGES PRODUCED IN THE ACIDITY OF MILK CONTAINING BROM-CRESOL-PURPLE,
BY DAYS

	1	2	3	4	5	7	10	14	21	30	60	90
<i>B. sanguinarum</i>	1	+	+c-	..	..	—	..	..	..	—	..	..
	2	+	+c-	..	..	—	..	..	..	—	..	..
	3	+	+c-	..	..	—	..	..	..	—	..	..
	11	+c-	..	..	..	—	..	..	..	—	..	..
	12	+c-	..	..	Nc-	—	..	..	..	—	..	..
	13	+	..	..	+	—	..	..	..	—	..	..
	14	+c-	..	..	Nc-	—	..	..	..	—	..	..
	15	+	..	..	+	Nc-	—	..	..	—	..	..
	40	+c-	..	..	Nc-	—	..	..	..	—	..	..
	41	+c-	..	..	..	—	..	..	..	—	..	..
	42	+c-	..	..	..	—	..	..	..	—	..	..
	47	+c-	..	..	Nc-	—	..	..	..	—	..	..
	50	+	..	..	..	..	..	..	..	+	—	..
	51	+c-	..	..	..	—	..	..	..	—	..	..
	52	+	..	+c-	..	Nc-	—	..	..	—	..	..
	53	+	..	..	..	..	..	..	..	+	—	..
	54	+c-	..	..	..	—	..	..	..	—	..	..
	55	+c-	..	..	..	..	..	..	..	—	..	..
	88	+	+c-	..	..	..	..	..	..	—	..	..
	56	+	..	Nc-	—	..	..	..	..	—	..	..
	57	+	..	..	..	..	..	..	..	—	..	..
<i>B. avisepticus</i>	44	+	+	..	..	..	..	..	..	+	+	+
	45	+	+	..	..	..	..	..	..	+	+	+
	46	+	+	..	..	..	..	..	..	+	+	+
	48	+	+	..	..	..	..	..	..	+	+	+
	49	+	+	..	..	..	..	..	..	+	+	+
	71	+	+	..	..	..	..	..	..	+	..	..
	72	+	+	..	..	..	..	..	..	+	..	..
<i>B. pullorum</i>	4	+	+c-	..	..	..	..	..	..	—	..	..
	5	+	+c-	..	..	..	—	..	..	—	..	..
	6	+	..	..	..	..	..	..	..	+	—	..
	7	+	..	..	..	..	..	..	..	+	—	..
	8	+	..	..	..	..	..	..	..	+	—	..
	9	+	..	..	..	..	..	..	..	—	..	..
	10	+	..	..	..	..	..	..	..	+	—	..
	16	+	..	..	..	..	..	..	..	—	..	..
	18	+	..	..	..	—	..	..	..	—	..	..
	19	+	+c-	..	—	..	..	..	..	—	..	..
	20	+c-	..	..	—	..	..	..	..	—	..	..
	21	+	..	..	..	..	..	..	..	—	..	..
	22	+	..	..	..	..	..	+c-	—	—	..	..
	23	+	..	..	..	..	+c-	—	..	—	..	..
	24	+c-	..	..	—	..	..	..	..	—	..	..
	25	+	..	..	..	..	..	—	..	—	..	..
	26	+	..	..	..	..	..	..	..	—	..	..
	27	+	+c-	..	Nc-	—	..	..	..	—	..	..
	28	+	..	+c-	Nc-	—	..	..	..	—	..	..
	29	+	..	..	..	..	..	..	..	+	—	..
	30	+	..	..	..	..	..	—	..	..	..	..
	31	+	..	..	..	..	..	..	..	N	—	..
	32	+	..	..	..	+c-	..	—	..	—	..	..

Symbols: + = acid.

+c- = body of tube acid and cream ring alkaline.

Nc- = body of tube neutral and cream ring alkaline.

— = alkaline reaction thruout medium.

In my own work the medium used in determining the fermentative reactions has been standard nutrient broth made from meat extract to which was added 0.5% of the carbohydrate to be tested. Andrade's indicator was used as the indicator of acid production in some of the earlier tests but brom-cresol-purple has been used in all the later tests. The indicator was added

to the medium which was then placed in tubes containing inverted vials. The medium was sterilized by heating in the autoclave for 10-15 minutes at 10 lbs. of pressure.

Some preliminary tests were made in which the amount of acid produced was determined by titration. But since this required considerable amounts of the medium in order to make observations at intervals over a period of several days and gave no information of importance other than was given by the indicator method, it has been discontinued.

The fermentative reactions are summarized in tables 4 and 5.

TABLE 4
ACID AND GAS PRODUCTION IN THE CARBOHYDRATES BY *B. PULLORUM*

Acid and Gas Positive	Acid and Gas Negative		Gas Variable and Acid Positive
Dextrose	Lactose	Erythrit	Xylose
Levulose	Saccharose	Dextrin	Sorbit
Galactose	*Maltose	Dulcite	
Mannite	Raffinose	Inosite	
Mannose	Inulin	Salicin	
Arabinose			
Rhamnose			

* Some strains of *B. pullorum* apparently ferment maltose, and *B. avisepticus* produces a very slight amount of acid sometimes.

TABLE 5
ACID PRODUCTION IN THE CARBOHYDRATES BY *B. SANGUINARIUM* AND *B. AVISEPTICUS*

<i>B. sanguinarium</i>		<i>B. avisepticus</i>	
Positive	Negative	Positive	Negative
Dextrose	Lactose	Dextrose	Lactose
Levulose	Saccharose	Levulose	Rhamnose
Galactose	Raffinose	Galactose	†Maltose
Mannite	Inulin	Mannite	
Mannose	Erythrit	Mannose	Dulcite
Arabinose	Inosite	Arabinose	
Rhamnose	Salicin		
Dulcite	†Sorbit	Sorbit	
Maltose		Saccharose	
Xylose		Xylose	
*Dextrin			

* Only a very slight amount of acid was formed by some strains, which made it practically impossible to distinguish from *B. pullorum* by the use of this carbohydrate alone.

† Acid production in sorbite varies with different strains as will be shown in a subsequent table.

‡ Some strains of *B. pullorum* apparently ferment maltose, and *B. avisepticus* produces a very slight amount of acid sometimes.

The fermentative reactions, however, are not as uniform as tables 4 and 5 indicate. In some cases it is rather difficult to differentiate strains of *B. pullorum* from *B. sanguinarium* by their fermentative capacities.

It is generally considered that maltose fermentation differentiates between *B. pullorum* and *B. sanguinarium*. Altho Krumwiede and

Kohn have observed that certain strains of *B. pullorum* produce a slight amount of acid in maltose after several days' incubation, they suggest that this is probably due to hydrolysis rather than any latent ability to ferment this carbohydrate. In my own work some strains of *B. pullorum* have produced a slight amount of acid after several days' incubation. This does not appear to be due to hydrolysis alone, since those strains which produce acid after several days' incubation do not form acid sooner in tubes which have been incubated for 3 weeks than in those freshly made up. Strains 20 and 32 have produced acid and gas in maltose somewhat irregularly. Subcultures of colonies picked from plates of these two strains are variable in their fermentation of maltose. In other respects these strains are typical. There also appears to be some variation in the fermentation of maltose by *B. sanguinarium*. Two strains have not fermented maltose promptly. They have fermented it only slightly after 10 days' incubation. The reaction in maltose and a few other carbohydrates, in which the reactions are quite variable, are given in table 6.

Dulcite is not fermented by any strain of *B. pullorum*, while all strains of *B. sanguinarium* but one ferment it promptly; Strain 14 ferments it rather slowly. There appears to be less variation in the fermentative action of these avian strains on dulcite than in any other carbohydrate.

Dextrin is fermented only slightly by most strains of *B. sanguinarium* and is not fermented at all by certain strains at different times. On the other hand, some strains of *B. pullorum* appear to ferment this carbohydrate slightly. Krumwiede and Kohn²⁴ have also observed that the fermentation of dextrin by *B. sanguinarium* is irregular. They believe that the results depend on the constituents of the sample, since other members of the paratyphoid-enteritidis group varied in a similar manner.

Acid is uniformly, tho slowly, produced in sorbite by *B. pullorum*, but the ability to form gas varies. *B. sanguinarium* is irregular in its action on sorbite. Some strains do not produce acid in 30 days, and in no case is acid formed before 5-7 days. The strains of *B. avisepticus* ferment this carbohydrate promptly.

Rhamnose is promptly fermented by all strains of *B. pullorum*, altho the gas production is somewhat irregular. The inability of *B. sanguinarium* to form acid promptly in rhamnose is quite uniform with all strains and may serve in a measure to differentiate between

B. sanguinarium and *B. pullorum*. The strains of *B. avisepticus* have not fermented this carbohydrate.

The chief fermentative differences between *B. pullorum* and *B. sanguinarium* are:

1. The production of gas by most strains of *B. pullorum* in several of the carbohydrates and the inability of *B. sanguinarium* to produce gas in any carbohydrate.

2. The fermentation of dulcitol and maltose by *B. sanguinarium* and the inability of *B. pullorum* to ferment these carbohydrates. A few strains of *B. pullorum* attack maltose slightly and two strains of *B. sanguinarium* produce acid very slowly in this sugar. Also Strains 20 and 32 of *B. pullorum* irregularly produce acid and gas in maltose.

3. The strains of *B. sanguinarium* studied ferment rhamnose slowly while the strains of *B. pullorum* ferment it promptly.

4. Some strains of *B. sanguinarium* do not produce acid in sorbitol, while all strains of *B. pullorum* produce acid.

AGGLUTINATION

The agglutinative relationship of these organisms to each other and to *B. typhosus* was first observed by Smith and Ten Broeck.¹ They also observed that these organisms were not agglutinated by serums of rabbits immunized toward the different types of *B. dysenteriae*, and to *B. cholerae suis*. Rettger and Koser²⁸ and Krumwiede and Kohn have also observed the close agglutinative relationship of *B. sanguinarium* and *B. pullorum* to *B. typhosus*. Pfeiler and Roepke⁷ have observed that an immune serum for the fowl typhoid organism did not agglutinate *B. typhosus* or *B. suispestifer* but agglutinated *B. paratyphosus* A and B, *B. enteritidis* and *B. psittacosis* in dilutions as high as 1:400.

In my work all determinations have been made by mixing 1 cc of the various dilutions of the serum in small tubes, with 1 cc of a 24-hour agar slant growth suspended in sufficient salt solution to give a uniform turbidity. The mixtures were incubated for 2 hours at 37 C., then placed in the ice chest for about 20 hours, when readings were made.

Agglutination tests with serum of rabbits immunized toward Strains 4, 7, 9, 19, 22 and 31 of *B. pullorum*, and Strains 2, 3, 11, 40 and 47 of *B. sanguinarium* have not shown any agglutinative differences between *B. pullorum* and *B. sanguinarium*. The strains with which I have worked have not been agglutinated when treated with a 1:250 dilution of serums from rabbits immunized toward typical strains of *B. paratyphosus* A and B, *B. suispestifer*, *B. abortus equinus*, and the Shiga and Flexner types of *B. dysenteriae*.

The agglutinative relation to *B. typhosus*, which has been observed by other investigators, has been observed in my own work. The

immune serum for *B. typhosus* 189—a stock culture—has not, as a rule, agglutinated the avian strains in as high dilutions as has that for *B. typhosus* “Hopkins,” when the titer of the serums was the same. All strains of *B. pullorum* and *B. sanguinarium* have been tested with these two serums. Some typical reactions are given in table 7.

TABLE 6
ACID AND GAS PRODUCTION IN BROTH CONTAINING 0.5% OF THE CARBOHYDRATE AND 1% OF A 0.5% SOLUTION OF BROM-CRESOL-PURPLE

Strain	Dextrose		Maltose		Arabinose		Rhamnose		Sorbitol		Xylose		Dulcitol	
	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
<i>B. sanguinarium</i> 1	+	—	+	—	+	—	+3	—	+10	—	+	—	+	—
2	+	—	+	—	+	—	+3	—	+7	—	+	—	+	—
3	+	—	+	—	+	—	+2	—	+10	—	+	—	+	—
11	+	—	+3	—	+	—	+3	—	+10	—	+	—	+	—
12	+	—	+	—	+	—	+3	—	+14	—	+2	—	+	—
13	+	—	+	—	+	—	+2	—	+10	—	+2	—	+	—
14	+	—	+21	—	+	—	+2	—	+21	—	+	—	+14	—
15	+	—	+	—	+	—	+2	—	+21	—	+	—	+	—
40	+	—	+	—	+	—	+3	—	—	—	+	—	+	—
41	+	—	+	—	+	—	+3	—	+21	—	+	—	+	—
42	+	—	+	—	+	—	+2	—	+21	—	+	—	+	—
47	+	—	+	—	+	—	+3	—	+14	—	+	—	+	—
									or —					
50	+	—	+14	—	+	—	+5	—	—	—	+	—	+	—
51	+	—	+	—	+	—	+3	—	—	—	+	—	+	—
52	+	—	+	—	+	—	+5	—	+10	—	+	—	+	—
53	+	—	+	—	+	—	+7	—	+21	—	+	—	+	—
54	+	—	+	—	+	—	+5	—	+21	—	+	—	+	—
55	+	—	+	—	+	—	+10	—	—	—	+	—	+	—
88	+	—	+	—	+	—	+5	—	+21	—	+	—	+	—
<i>B. pullorum</i> 4	+	+	+30	—	+	+	+	+	+6	+	+	+2	—	—
5	+	+	+21	—	+	+2	+	+	+7	+14	+	+2	—	—
6	+	+	—	—	+	—	+	+2	+10	—	+	—	—	—
7	+	+	—	—	+	—	+	+2	+10	—	+	—	—	—
8	+	+	—	—	+2	—	+	—	+7	—	+	—	—	—
9	+	+	—	—	+	—	+	+2	+10	—	+	+6	—	—
10	+	+2	±30	—	+	—	+	+4	+10	—	+6	—	—	—
16	+	+2	—	—	+2	+2	+	—	+7	—	+6	—	—	—
17	+	+	+10	—	+	+	+	+	+10	—	+	+2	—	—
19	+	+	+30	—	+	+	+	—	+7	+	+	+2	—	—
20	+	+	+21	±21	+	+2	+	+	+7	+7	+	+2	—	—
21	+	+	—	—	+	+3	+	+	+6	—	+	—	—	—
22	+	+	+30	—	+	—	+	+2	+6	+14	+2	+6	—	—
23	+	+	—	—	+	—	+	—	+10	—	+	—	—	—
24	+	+	—	—	+	+2	+	+	+10	+14	+	+3	—	—
25	+	+	—	—	+	—	+	+2	+10	—	+	—	—	—
26	+	+	+30	—	+2	—	+	+3	+10	—	+	+3	—	—
27	+	+	—	—	+	—	+	+	+7	—	+	+2	—	—
28	+	+2	—	—	+	+2	+	+3	+10	+30	+3	—	—	—
29	+	+	+30	—	+	+2	+	+2	+10	—	+2	—	—	—
30	+	+2	—	—	+	—	+	+2	+10	—	+2	—	—	—
31	+	+	—	—	+	—	+	—	+10	+10	+	+6	—	—
32	+	+	+3	+3	+	+	+	+	+10	+10	+	+3	—	—
<i>B. avisepticus</i> 44	+	—	±	—	+	—	—	—	+2	—	+5	—	—	—
45	+	—	±	—	+	—	—	—	+2	—	+7	—	—	—
46	+	—	±	—	+	—	—	—	+2	—	+5	—	—	—
48	+	—	±	—	+	—	—	—	+	—	±7	—	—	—
49	+	—	±	—	+	—	—	—	+2	—	±7	—	—	—

Symbols: + = acid or gas production in 24 hours.

— = no acid or gas production in 30 days.

A figure following a (+) indicates the number of days after inoculation when acid or gas was first observed.

± = very slight acid production or irregularity in acid production.

TABLE 7

AGGLUTINATION OF *B. PULLORUM* AND *B. SANGUINARIUM* WITH SERUMS FROM RABBITS IMMUNIZED TO *B. TYPHOSUS*

Serum of Rabbit Treated with <i>B. typhosus</i> Hopkins Titer 1:5000				Serum of Rabbit Treated with <i>B. typhosus</i> 189 Titer 1:5000			
Dilution of Serum				Dilution of Serum			
500	1000	2500	5000	500	1000	2500	5000
++	++	+	—	++	+	—	—
++	++	++	+	++	++	+	—
++	++	++	+	+++	++	++	+
++	++	++	+	++	+	—	—
++	++	++	+	+++	++	+	+
++	++	++	+	+++	++	+	—

= complete agglutination.
 = marked agglutination.
 = slight agglutination.
 = no agglutination.

The agglutinative relation between *B. enteritidis* and these organisms has not been reported by other investigators, except that Pfeiler and Koepke observed that immune serums for two strains of *B. enteritidis* with a titer of 1:10,000 and 1:16,000, respectively, agglutinated the "bacillus of fowl typhoid" in a dilution as high as 1:800. In my work, immune serums toward two strains of *B. enteritidis* (Jordan's strains 52 and 205) have agglutinated all the avian strains. Some strains of *B. pullorum* and *B. sanguinarium*, however, have been only slightly agglutinated in dilutions of 1:250 when the titer was 1:10,000. In general, the serum of the rabbit immunized to *B. enteritidis* (52) has agglutinated the avian strains in higher dilutions than the immune serum for *B. enteritidis* (205). Some typical reactions are given in table 8.

TABLE 8

AGGLUTINATION OF *B. PULLORUM* AND *B. SANGUINARIUM* WITH SERUMS FROM RABBITS IMMUNE TO *B. ENTERITIDIS*

Serum of Rabbit Treated with <i>B. enteritidis</i> 52 Titer 1:16,000				Serum of Rabbit Treated with <i>B. enteritidis</i> 205 Titer 1:16,000			
Dilution of Serum				Dilution of Serum			
500	1000	2500	5000	100	250	500	1000
+++	++	+	—	+++	++	—	—
+++	++	+	—	+++	++	+	—
+++	++	+	—	+++	++	++	+
+++	++	+	—	+++	++	+	—
+++	++	+	—	+++	++	+	—
+++	++	+	—	+++	++	+	—

Serums are the same as those in Table 7.

The agglutinative powers of serums of rabbits immunized toward several avian strains have been tested. The serums of rabbits, immunized toward Strains 4, 7, 9, 19, 22 and 31 of *B. pullorum*, and Strains 2, 3, 11, 40 and 47 of *B. sanguinarium* have in general reacted alike in all respects. These serums have not agglutinated in 1:500 dilution typical strains of *B. paratyphosus* A and B, *B. suipestifer*, *B. dysenteriae* Flexner, *B. dysenteriae* Shiga, *B. coli*, *B. proteus vulgaris*, and *B. avisepticus*. Strains of *B. typhosus*, *B. enteritidis*, and *B. abortus equinus*, however, have been agglutinated by immune *B. pullorum* or *B. sanguinarium* sera.

Nine strains of *B. enteritidis* have been tested with several of these immune serums. A few strains of *B. enteritidis* have been agglutinated in as high dilutions as some of the avian strains. It thus appears that *B. enteritidis* is agglutinated in higher dilutions by these sera, than *B. sanguinarium* or *B. pullorum* is agglutinated by immune *B. enteritidis* serum when the titer of the serums is the same. The agglutinative action, on *B. enteritidis*, of the serum of a rabbit immunized to *B. pullorum*, is given in table 9.

TABLE 9
AGGLUTINATION OF *B. ENTERITIDIS* WITH IMMUNE SERUM TO *B. PULLORUM* (9).
TITER 1:10,000

	1:500	1:1000	1:2500	1:5000	1:10,000
<i>B. sanguinarium</i> 3.....	+++	+++	++	+	—
<i>B. pullorum</i> 9.....	+++	+++	++	++	+
<i>B. enteritidis</i> 50.....	+++	++	+	—	—
51.....	+++	++	+	—	—
52.....	+++	++	+	—	—
53.....	+++	++	+	—	—
153.....	+++	+++	++	+	—
204.....	+++	++	++	—	—
205.....	+++	++	—	—	—
207.....	+++	++	+	—	—
228.....	+++	++	+	—	—

The agglutination of *B. typhosus* with serums of rabbits immunized to *B. sanguinarium* or *B. pullorum* was first observed by Smith and Ten Broeck. Their results have since been confirmed by Rettger and Koser, and Krumwiede and Kohn. Pfeiler and Roepke, however, have observed that a serum, with a titer of 8,000, from a rabbit immunized toward the "bacillus of fowl typhoid" did not agglutinate *B. typhosus* in a dilution of 1:100. In the present study three strains of *B. typhosus* have been agglutinated in high dilutions with serums of rabbits immune to the avian strains. The stock culture *B. typhosus*

189 generally did not agglutinate as well as the other two strains. Typical results are given in table 10.

TABLE 10
AGGLUTINATION REACTION OF *B. PULLORUM* (19) SERUM. TITER 1:5000

Strain	Dilution of Serum						
	100	250	500	1,000	2,500	5,000	10,000
<i>B. sanguinarium</i> 1.....			+++	++	+	—	—
41.....			+++	+	+	—	—
47.....				+++	++	+	—
<i>B. pullorum</i> 10.....			+++	+++	++	+	—
22.....			+++	+++	++	+	+
31.....			+++	+++	+	—	—
<i>B. typhosus</i> Hopkins.....			+++	++	++	+	—
189.....			++	++	—	—	—
197.....			+++	++	++	—	—
<i>B. enteritidis</i> 52.....			+++	+++	+	+	—
205.....			+++	++	+	—	—
228.....			++	+	—	—	—
<i>B. abortus equinus</i> 192.....			+++	++	+	—	—
193.....			+++	+++	+	—	—
196.....			+++	+++	++	+	—
<i>B. avisepticus</i> 44.....	+	—	—	—	—	—	—
46.....	—	—	—	—	—	—	—
48.....	—	—	—	—	—	—	—
<i>B. paratyphosus</i> A 4.....	—	—	—	—	—	—	—
<i>B. paratyphosus</i> B 12.....	+	—	—	—	—	—	—
<i>B. suispestifer</i> 118.....	—	—	—	—	—	—	—

TABLE 11
AGGLUTINATION REACTION OF *B. SANGUINARIUM* (40) SERUM. TITER 1:10,000

Strain	Dilution of Serum						
	100	250	500	1,000	2,500	5,000	10,000
<i>B. sanguinarium</i> 2.....			+++	+++	+++	++	+
14.....			+++	++	+	—	—
88.....			+++	+++	++	+	—
<i>B. pullorum</i> 8.....			+++	+++	++	+	—
22.....			+++	+++	++	++	+
31.....			+++	++	++	+	—
<i>B. typhosus</i> Hopkins.....			+++	+++	++	+	—
189.....			+++	+++	++	—	—
197.....			+++	+++	++	+	—
<i>B. enteritidis</i> 52.....			+++	++	+	—	—
153.....			+++	++	++	+	—
206.....			+++	++	—	—	—
<i>B. abortus equinus</i> 192.....			+++	++	++	+	—
195.....			+++	++	++	+	—
196.....			+++	+++	++	+	+
<i>B. avisepticus</i> 44.....	—	—	—	—	—	—	—
45.....	—	—	—	—	—	—	—
49.....	—	—	—	—	—	—	—
<i>B. paratyphosus</i> A 4.....	—	—	—	—	—	—	—
<i>B. paratyphosus</i> B 12.....	—	—	—	—	—	—	—
<i>B. suispestifer</i> 118.....	—	—	—	—	—	—	—

In studying the agglutinative relation of the avian strains to *B. abortus-equinus* it was observed that the serums of rabbits immunized to *B. pullorum* or *B. sanguinarium* contained agglutinins for five strains of *B. abortus-equinus*. Rettger and Koser, however, observed that a strain of *B. abortus* used as a control was not agglutinated by

immune *B. pullorum* serum. In my work it was noted that *B. pullorum* and *B. sanguinarium* were not agglutinated by the serum of a rabbit immunized toward *B. abortus-equinus*.

Some typical agglutinative reactions of serums from rabbits immunized to *B. pullorum* are given in Table 10.

Results similar to those in the preceding tables have been observed with serums of rabbits immunized toward other strains of *B. sanguinarium* and *B. pullorum*, but it does not seem necessary to give tables. The agglutination of *B. typhosus*, *B. enteritidis*, and *B. abortus-equinus* with such serums is a rather remarkable case of cross agglutination. These results suggest that *B. enteritidis* is more clearly related, at least antigenically, to these organisms and to *B. typhosus* than other members of the paratyphoid-enteritidis group.

ABSORPTION TESTS

In order to test further the antigenic relation of these organisms to *B. typhosus*, *B. enteritidis*, and *B. abortus-equinus*, absorption tests were made. The tests were carried out by suspending in about 25 cc of isotonic salt solution, the 24-hour growth on an agar slant, whose surface area was approximately 60 sq. cm. The suspension was centrifugalized and the supernatant liquid poured off. In some tests the organisms were washed a second time but this did not change the results. The organisms were then suspended in 5 cc of salt solution and to this suspension was added 5 cc of a 1:25 dilution of the serum to be tested. This mixture was placed in a small flask and incubated for 3 hours at 37 C. and then placed in the ice box for 20-48 hours. They were then centrifugalized and the remaining titer for the various organisms determined.

TABLE 12
ABSORPTION TESTS WITH SERUM OF RABBIT TREATED WITH *B. TYPHOSUS* 189,
TITER 1:20,000

Exhausted with	Original Titer	Remaining Titer for		
		<i>B. typhosus</i> Hopkins	<i>B. pullor-</i> um 22	<i>B. sanguin-</i> arium 47
<i>B. typhosus</i> 189.....	20,000	<100	<100	<100
<i>B. sanguinarium</i> 47.....	20,000	10,000	<100	250
<i>B. pullorum</i> 22.....	20,000	20,000	100	250
<i>B. enteritidis</i> 52.....	100	20,000	<100	100

In absorption tests with typhoid immune serum it was observed that *B. sanguinarium* or *B. pullorum* removed the agglutinins for these avian strains but removed only a small percentage or none of the agglutinins for *B. typhosus*. Smith and Ten Broeck obtained similar results. *B. enteritidis* also removed a large percentage or all of the agglutinins for the avian strains. Typical results are given in table 12.

With the serums of rabbits immunized toward *B. enteritidis*, it was observed that the avian strains did not remove the agglutinins for *B. enteritidis*. The three strains of *B. typhosus*, which I have used, removed the agglutinins for the avian strains from these sera. table 13 shows typical reactions.

TABLE 13
ABSORPTION TESTS WITH SERUM OF RABBIT TREATED WITH *B. ENTERITIDIS* (52)

Exhausted with	Original Titer	Remaining Titer for		
		<i>B. enteriti- dis</i> 52	<i>B. sanguin- arium</i> 47	<i>B. pullor- um</i> 22
<i>B. enteritidis</i> 52.....	10,000	100	—	—
<i>B. sanguinarium</i> 47.....	2,500	10,000	—	—
<i>B. pullorum</i> 22.....	2,500	10,000	—	—
<i>B. typhosus</i> Hopkins.....	<100	10,000	100	—

From the results indicated in the preceding tables it appears that *B. typhosus* and *B. enteritidis* have a certain antigenic relation to each other which is common to the avian strains. Thus the agglutinins for the avian strains, which have been studied in this respect, are removed from typhoid immune serum by *B. enteritidis*, and from immune *B. enteritidis* serum by *B. typhosus*. Further antigenic relations between *B. typhosus* and *B. enteritidis* are shown in absorption tests with serums of rabbits immunized toward *B. pullorum* or *B. sanguinarium*.

Absorption tests with serums of rabbits immunized toward *B. pullorum* (9) and (22) and *B. sanguinarium* (40) are given in table 14, 15 and 16.

Results similar to those tabulated in these tables have been obtained with serums of rabbits immunized toward *B. pullorum* 19 and 31, and toward *B. sanguinarium* 47 and 11. According to the tables the strains of *B. typhosus* and *B. enteritidis* remove a small percentage of the agglutinins for strains of *B. pullorum* or *B. sanguinarium* from serums of rabbits immunized with some strains of *B. pullorum* or *B. sanguinarium*. Smith and Ten Broeck observed that their strains of *B. typhosus* removed the agglutinins for the avian strains from serums of rabbits immunized toward *B. sanguinarium* or *B. pullorum*. I also noted in some earlier tests that *B. typhosus* or *B. enteritidis* irregularly removed all the agglutinins from such serums. In the earlier tests the organisms were not washed, and it was observed that those growths on slant agar surfaces, which had the most water of con-

densation, removed a large percentage or all the agglutinins. The results were quite irregular with unwashed organisms, but with washed organisms the results were much more regular. The agar on which the organisms were grown had a reaction of +1 to phenolphthalein.

TABLE 14

ABSORPTION TESTS WITH SERUM OF RABBIT TREATED WITH *B. PULLORUM* (9)

Exhausted with	Original Titer	Remaining Titer for			
		<i>B. pullorum</i> 22	<i>B. typhosus</i> Hopkins	<i>B. enteritidis</i> 52	<i>B. abortus equinus</i> 196
<i>B. pullorum</i> 9.....	5,000	100	100	100	100
<i>B. typhosus</i> Hopkins....	5,000	1,600	100	800	200
<i>B. enteritidis</i> 52.....	2,500	2,500	2,500	100	800
<i>B. abortus equinus</i> 196...	2,500	5,000	2,500	800	200

TABLE 15

ABSORPTION TESTS WITH SERUM OF RABBIT TREATED WITH *B. PULLORUM* (22)

Exhausted with	Original Titer	Remaining Titer for				
		<i>B. sanguinarium</i> 47	<i>B. pullorum</i> 22	<i>B. typhosus</i> Hopkins	<i>B. enteritidis</i> 52	<i>B. abortus equinus</i> 196
<i>B. pullorum</i> 9.....	5,000	250	500	250	250	<100
<i>B. sanguinarium</i> 3.....	10,000	100	100	<100	<100	<100
<i>B. typhosus</i> Hopkins.....	5,000	5,000	2,500	100	2,500	5,000
<i>B. enteritidis</i> 52.....	4,000	10,000	5,000	1,000	100	2,500
<i>B. abortus equinus</i> 196.....	5,000	10,000	5,000	5,000	2,500	250

TABLE 16

ABSORPTION TESTS WITH SERUM OF RABBIT TREATED WITH *B. SANGUINARIUM* (40)

Exhausted with	Original Titer	Remaining Titer for				
		<i>B. sanguinarium</i> 47	<i>B. pullorum</i> 22	<i>B. typhosus</i> Hopkins	<i>B. enteritidis</i> 52	<i>B. abortus equinus</i> 196
<i>B. pullorum</i> 22.....	10,000	100	100	<100	100	<100
<i>B. sanguinarium</i> 47.....	10,000	<100	<100	<100	<100	<100
<i>B. typhosus</i> Hopkins.....	5,000	10,000	5,000	100	1,000	1,000
<i>B. enteritidis</i> 52.....	5,000	10,000	5,000	1,000	250	1,000
<i>B. abortus equinus</i> 196.....	5,000	10,000	5,000	2,500	2,500	100

B. typhosus apparently removed a large percentage of the agglutinins from such serums for *B. enteritidis*, and on the other hand, *B. enteritidis* appeared to remove a large percentage of the agglutinins from these serums for *B. typhosus*. *B. abortus-equinus* did not remove agglutinins for other organisms to any great extent.

While absorption tests are not necessary to differentiate *B. abortus equinus* or *B. enteritidis* from these avian strains, yet it was thought that these might throw some light on the antigenic properties of the avian types. From absorption tests as well as from agglutination tests it appears that *B. enteritidis* occupies an intermediate position between *B. typhosus* and *B. abortus-equinus* in its relation to the avian strains. These absorption tests also indicate an antigenic relation between *B. typhosus* and *B. enteritidis*.

PATHOGENESIS

A high percentage of fatal infections following the feeding of *B. pullorum* to rabbits, guinea-pigs, and cats has been reported by Rettger, Hull and Sturges. Smith and Ten Broeck have observed rapidly fatal results following the intravenous injection of broth cultures of *B. sanguinarium* into rabbits. The latter investigators considered the sudden onset of symptoms to be due to a toxin, since if animals recovered from the immediate effects they died a few days later from an infection. In the present study, pathogenesis has been tested by feeding and intraperitoneal inoculations.

FEEDING EXPERIMENTS

In the feeding experiments the cultures have been grown on agar slants, in milk and in meat infusion broth. The broth and milk cultures were incubated from 1-5 days, before they were fed to the animals. The longer incubation period was used to determine the development of toxic substances which might show their presence by producing symptoms of food poisoning when ingested. The method of administering the test organisms varied. In some instances especially in feeding rabbits and guinea-pigs large amounts, water suspensions of 24-hour slant agar cultures were mixed with finely ground carrots, while at other times these suspensions or broth cultures were mixed with milk. In some experiments with cats, rats, and mice finely ground meat was employed which had been sterilized and inoculated with the organisms to be tested and incubated for 24-48 hours. In feeding the birds the suspensions of the cultures were added to dried bread crumbs. The amounts of cultures fed varied from 1-12 agar slant cultures, or in the case of broth or milk cultures from 5-30 c.c. In some cases, where counts were made by plating on agar, approximately 100 billion bacteria were fed.

Different strains of *B. pullorum* have been fed to 7 guinea-pigs weighing between 200 and 350 gm.; 6 rabbits weighing between 1,500 and 2,500 gm.; 2 young rabbits weighing about 700 gm.; 5 kittens weighing between 620 and 925 gm.; 6 mice; 4 rats; 2 cats; 6 sparrows; 5 young pigeons less than 48 hours of age; 2 adult pigeons; 10 chicks about 2 weeks of age, and 4 young hens about 4 months old. Cultures of *B. sanguinarium* have been fed in a similar manner to about the same number of animals.

No symptoms of disease were observed following the ingestion of these organisms, except that one guinea-pig, which was fed 15 cc of a 48-hour milk culture of *B. pullorum* (9), appeared sick 4 days after it was fed the culture and died the following day. No lesions, other than a slight congestion of the abdominal viscera, were observed at necropsy. *B. pullorum* was isolated in pure culture from the heart blood and liver.

Cats, which were fed sparrows, or rabbits that had died from an infection with *B. pullorum* or *B. sanguinarium*, showed no symptoms of disease. Also the freshly isolated strains, *B. pullorum* (31) fed within 2 weeks after isolation from a dead chick, and *B. sanguinarium* (47 and 57) fed 4-7 days after isolation, did not produce any injurious effect when fed to rabbits, guinea-pigs, mice, young and adult pigeons and sparrows. Chickens apparently are not very susceptible to laboratory cultures of these avian strains.

Rettger and others consider that chickens more than 5 days old are generally not readily susceptible to *B. pullorum*. Jones,¹² Rettger,² and Hadley,¹³ however, have observed fatal infections in adult fowls with this organism. Pfeiler and Roepke⁷ working with *B. sanguinarium* have observed that chickens cannot regularly be infected by feeding cultures of this organism, but feeding the viscera of fowls dead of the disease generally produces the disease. They also observed that turkeys, guineas, and peacocks contracted the disease under natural conditions, but ducks and geese did not. They succeeded, however, in producing a fatal infection in a duck by inoculation. One of the strains of *B. sanguinarium* (41) which I have studied was isolated from a wild duck, which with several hundred others had apparently died from an infection with this organism.

From the feeding experiments it appears that rabbits, guinea-pigs, cats, rats, mice, sparrows, pigeons and chickens are not susceptible to laboratory cultures of *B. pullorum* or *B. sanguinarium* when administered by mouth.

INOCULATION EXPERIMENTS

Inoculations of cultures of these organisms into laboratory animals have not given uniform results with the different investigators. Smith and Ten Broeck observed that 0.3-0.5 cc of a 24-hour broth culture of *B. sanguinarium* produced a fatal infection in rabbits when injected intravenously. Taylor,¹⁷ however, observed that 1 cc of a 24-hour broth culture of this organism did not produce death in rabbits when injected intraperitoneally. Guinea-pigs have been found by several investigators to be quite resistant to injections of cultures of *B. sanguinarium*. Pfeiler and Roepke state that rats, cats, and dogs are resistant to inoculations with this organism. Smith and Ten Broeck observed that 2 cc of a 24-hour broth culture of the fowl typhoid organism did not prove fatal to a chicken inoculated intravenously.

Comparatively few inoculation experiments with *B. pullorum* have been made. Rettger² and Gage consider the rabbit very susceptible to inoculations with *B. pullorum*.

TABLE 17
INOCULATION EXPERIMENTS WITH MICE

Animals	Strain	Condition and Amount of Culture	Results
2 mice	<i>B. sanguinarium</i> 2	0.5 c c of 24-hour broth culture to each	Affected slightly
1 mouse	<i>B. sanguinarium</i> 2	1 c c of 24-hour broth culture	*Found dead on next day
1 mouse	<i>B. sanguinarium</i> 47	0.5 c c of 24-hour broth culture isolated 5 days before injected	Slightly sick
1 mouse	<i>B. sanguinarium</i> 47	1 c c of 24-hour broth culture isolated 5 days before injected	*Died in 24 hours
2 mice	<i>B. pullorum</i> 9	0.5 c c of 24-hour broth culture to each	Slightly sick
2 mice	<i>B. pullorum</i> 5	0.5 c c of agar slant culture suspended in 10 c c salt solution	Slightly sick
1 mouse	<i>B. pullorum</i> 5	1 c c of agar cultures suspension	*Died after 18 hrs.
1 mouse	<i>B. pullorum</i> 7	0.5 c c of 24-hour broth culture	Slightly sick
1 mouse	<i>B. pullorum</i> 31	0.5 c c of 24-hour broth culture	Slightly sick
1 mouse	<i>B. pullorum</i> 31	1 c c of 24-hour broth culture	*Died on 4th day

* Organisms were recovered from heart blood and liver.

TABLE 18
INOCULATION EXPERIMENTS WITH GUINEA-PIGS

Animals	Strain	Condition and Amount of Culture	Results
1. Wt. 290 Gm.	<i>B. sanguinarium</i> 3	2 c c of 24-hour broth culture	*Died 20 hours later
2. Wt. 275 Gm.	<i>B. sanguinarium</i> 11	2 c c of 24-hour broth culture	*Died 18 hours later
3. Wt. 290 Gm.	<i>B. sanguinarium</i> 11	1 c c of 24-hour broth culture	Survived
4. Wt. 320 Gm.	<i>B. sanguinarium</i> 47	1 c c of 24-hour broth culture isolated 5 days before injected	Survived
5. Wt. 340 Gm.	<i>B. sanguinarium</i> 47	2 c c of 24-hour broth culture	Lost 60 Gm. in 3 days. Recovered
6. Wt. 400 Gm.	<i>B. sanguinarium</i> 11	2 c c of agar slant culture suspended in 10 c c salt	*Dead in 16 hours
7. Wt. 310 Gm.	<i>B. pullorum</i> 9	1 c c of 24-hour broth culture	Survived
8. Wt. 325 Gm.	<i>B. pullorum</i> 9	2 c c of 24-hour broth culture	Survived
9. Wt. 345 Gm.	<i>B. pullorum</i> 9	3 c c of 24-hour broth culture	*Dead in 48 hours
10. Wt. 310 Gm.	<i>B. pullorum</i> 10	1 c c of 48-hour broth culture	Survived
11. Wt. 370 Gm.	<i>B. pullorum</i> 10	2 c c of 48-hour broth culture	Survived
12. Wt. 350 Gm.	<i>B. pullorum</i> 16	2 c c of 24-hour broth culture	Survived
13. Wt. 330 Gm.	<i>B. pullorum</i> 16	3 c c of 24-hour broth culture	*Died in 4 days
14. Wt. 320 Gm.	<i>B. pullorum</i> 19	2 c c of 48-hour broth culture	*Died in 48 hours

* Organisms were recovered from heart blood and liver.

In my own work, broth cultures, and agar slant cultures suspended in 10 c c of salt solution, have been used. Inoculations were made intraperitoneally unless otherwise stated. Details of inoculation experiments are given in tables 17, 18, 19 and 20.

In addition to the experiments tabulated, white rats and pigeons were inoculated with 1-2.5 c c of 24-hour broth cultures of different strains of *B. pullorum* and *B. sanguinarium*. The rats were inoculated intraperitoneally

and the pigeons were injected intramuscularly. All the rats and pigeons survived the inoculations except that one pigeon died 4 days after being inoculated with 1.5 cc of a 24-hour broth culture of *B. sanguinarium* (47). This culture of *B. sanguinarium* had been isolated 5 days previously, from a hen which had died of fowl typhoid.

TABLE 19
INOCULATION EXPERIMENTS WITH RABBITS

Animals	Strain	Condition and Amount of Culture	Results
1. Wt. 2,580 Gm.	<i>B. sanguinarium</i> 1	1.5 cc of 24-hour broth culture	Little effect
2. Wt. 2,130 Gm.	<i>B. sanguinarium</i> 2	2 cc of 24-hour broth culture	Sick for 3 days. Recovered
3. Wt. 2,180 Gm.	<i>B. sanguinarium</i> 2	1.5 cc of 48-hour broth culture	*Died on 5th day
4. Wt. 1,275 Gm.	<i>B. sanguinarium</i> 47	1 cc of 24-hour broth culture. Isolated 5 days previously	No effect
5. Wt. 1,645 Gm.	<i>B. sanguinarium</i> 47	2.5 cc of 24-hour broth culture	*Died on 4th day
6. Wt. 1,780 Gm.	<i>B. pullorum</i> 5	2 cc of 24-hour broth culture	No effect
7. Wt. 2,250 Gm.	<i>B. pullorum</i> 7	2 cc of 24-hour broth culture	No effect
8. Wt. 2,360 Gm.	<i>B. pullorum</i> 9	2 cc of 24-hour broth culture	*Dead on 5th day
9. Wt. 1,520 Gm.	<i>B. pullorum</i> 9	1 cc of 24-hour broth culture	Survived
10. Wt. 1,075 Gm.	<i>B. pullorum</i> 22	1.5 cc of 24-hour broth culture	Little effect
11. Wt. 1,540 Gm.	<i>B. pullorum</i> 31	1.5 cc of 24-hour broth culture. Had been isolated about 2 weeks previously	Little effect
12. Wt. 2,270 Gm.	<i>B. sanguinarium</i> 56	2 cc of an agar slant culture suspended in 10 cc salt solution. Isolated about 2 weeks before	Recovered

* Organisms were recovered from heart blood and liver.

TABLE 20
INOCULATION EXPERIMENTS WITH SPARROWS

Sparrow No.	Strain	Amount and Condition of Culture	Results
1	<i>B. sanguinarium</i> 2	0.25 cc of 24-hour broth culture	Recovered
2	<i>B. sanguinarium</i> 11	0.25 cc of 24-hour broth culture	Recovered
3	<i>B. sanguinarium</i> 2	0.3 cc of 24-hour broth culture	Found dead after 4 days. Could not recover organisms
4	<i>B. sanguinarium</i> 11	0.3 cc of 24-hour broth culture	Recovered
5	<i>B. sanguinarium</i> 3	0.5 cc of 24-hour broth culture	*Died on 4th day
6	<i>B. pullorum</i> 7	0.25 cc of 24-hour broth culture	No effect
7	<i>B. pullorum</i> 9	0.3 cc of 24-hour broth culture	*Died 4 days later
8	<i>B. pullorum</i> 9	0.3 cc of 24-hour broth culture	*Died 4 days later
9	<i>B. pullorum</i> 10	0.25 cc of 24-hour broth culture	*Found dead after 4 days
10	<i>B. pullorum</i> 10	0.3 cc of 24-hour broth culture	*Died 3 days later
11	<i>B. pullorum</i> 29	1 cc of an agar slant growth suspended in 10 cc of salt solution	*Died 3 days later
12	<i>B. pullorum</i> 29	1 cc of the above suspension	*Found dead 4 days later

* The organisms were recovered from the heart blood and the liver of these animals.

Four young hens weighing between 840 and 1,350 gm. were inoculated intramuscularly with 2 cc, 4 cc, 5 cc, and 8 cc, respectively, of 5-day broth cultures of *B. sanguinarium*. Two cc of 24-hour broth cultures of *B. pullorum* (9 and 31) and *B. sanguinarium* (11 and 56) were also injected intramuscularly into hens weighing from 800 to 1,300 gm. A rooster weighing 1,875 gm. was inoculated with 2 cc of a 24-hour broth culture of *B. sanguinarium*

(57). This culture had been isolated 5 days previously from a hen that had died of fowl typhoid. All of the chickens recovered. The one that received 8 cc of the broth culture of *B. sanguinarium* (11) appeared sick for a few hours after inoculation.

From these experiments it appears that mice, rats, guinea-pigs, pigeons, sparrows, chickens and rabbits are quite resistant to laboratory cultures of these organisms. The rabbit is apparently the most susceptible. The amount necessary to kill adult rabbits varies considerably, but is generally between 2 and 3 cc of a 24-hour broth culture. It generally requires a similar amount to kill the guinea-pig. Rats and mice are quite resistant. Pigeons, sparrows and chickens were also quite resistant to the laboratory cultures of these organisms, altho a pigeon was more susceptible to a freshly isolated culture of *B. sanguinarium*. The sparrow appeared to be slightly more susceptible to *B. pullorum*. In general, the pathogenesis of these two avian strains is the same for the laboratory animals.

TOXIN PRODUCTION

Smith and Ten Broeck observed that the injection into rabbits of 2 cc of the bacterial free filtrates of 5-day broth cultures of *B. sanguinarium* or of *B. pullorum* regularly produced death in a few hours. They further observed that guinea-pigs were refractory toward this toxin. The medium which they found to be the most suitable for toxin production was freshly prepared sugar-free veal broth to which was added 0.1% of dextrose. Another factor, which they considered very important, was that the depth of the layer of fluid in which the cultures were grown should not be more than 2 cm. In my work a similar medium was used except that Armour's peptone was used instead of Witte's. Results of injections of bacterial free filtrate into rabbits and chickens are given in Tables 21 and 22.

Since the results of the inoculations of toxic filtrates into guinea-pigs and white mice were largely negative, it seems unnecessary to tabulate the results. The guinea-pigs used in these tests weighed between 225 and 325 gm. Four guinea-pigs, each injected with 2 cc of the toxic filtrate of different strains of *B. sanguinarium*, survived. Two guinea-pigs, of five injected with 2 cc of the toxic filtrate of different strains of *B. pullorum*, died within 48 hours. White mice in a few cases were killed by injecting 1 cc of the toxic filtrates, but in other cases they survived such injections.

TABLE 21
INTRAPERITONEAL INJECTIONS OF 5-DAY BOUILLON FILTRATES INTO RABBITS

Strain	Amount Injected, C O	Weight in Grams of Rabbit	Results
B. sanguinarium 11	2	1,980	Breathing very difficult after 2 hours. Died an hour later. Marked rigor 5 minutes after death. Liver and spleen congested
B. sanguinarium 11	2	2,635	Breathing very difficult 2 hours later. Dead in 4 hours. Hemorrhages in gastric mucosa
B. sanguinarium 47	2	1,720	Very sick an hour later. Died in 2 hours. Marked rigor few minutes after death. Hemorrhages in gastric mucosa and under endocardium
B. sanguinarium 47	2	1,740	Very sick for several hours. Lost 160 gm. in 3 days. Recovered
B. pullorum 19	1.8	2,315	Quite sick 2 hours later. Appeared better the next morning. Recovered
B. pullorum 19	2	2,150	Found dead 20 hours later. Hemorrhages in gastric mucosa and thymus
B. pullorum 24	1.5	2,175	Died in 4 hours. Hemorrhages in thymus and gastric mucosa. Marked rigor few minutes after death
B. pullorum 24	2	2,290	Quite sick for several hours. Lost 115 gm. in 24 hours. Recovered
B. pullorum 8	1	1,655	Slightly affected. Lost 30 gm. in 24 hours. Recovered
B. pullorum 8	2	1,690	Quite sick. Lost 130 gm. in 48 hours. Normal weight in 14 days
B. pullorum 9	1.5	1,720	Died 2 hours later. Hemorrhages in thymus and endocardium. Both kidneys showed chronic interstitial nephritis
B. pullorum 9	2	1,795	Found dead 10 hours later. Hemorrhages in thymus and gastric mucosa
B. pullorum 28	2	1,875	Dead after 15 hours. Gastric mucosa and thymus hemorrhagic
B. pullorum 28	1	1,675	Quite sick. Recovered
B. pullorum 25	1.5	1,615	Quite sick. Lost 145 gm. in 24 hours
B. pullorum 25	2	1,690	Recovered
B. pullorum 25	2	1,690	Died in 6 hours. Hemorrhages into gastric mucosa
B. pullorum 32	1.5	1,880	Slightly affected
B. pullorum 32	1.5	1,375	Quite sick. Lost 50 gm. in 24 hours
B. sanguinarium 3	2	1,890	Death occurred in 4 hours. Hemorrhages in gastric mucosa and pericardium
B. sanguinarium 41	2	2,240	Death in 5 hours. Hemorrhages in thymus and gastric mucosa
B. sanguinarium 31	2	1,745	Death in 2 hours. Hemorrhages in the thymus

TABLE 22
INTRAPERITONEAL INJECTIONS OF 5-DAY BOUILLON FILTRATES INTO CHICKENS

Strain	Amount Injected, C O	Weight in Grams of Chicken	Results
B. sanguinarium 3	5	1,215	Slightly sick for a few hours
B. sanguinarium 11	8	1,560	Quite sick for few hours
B. pullorum 9	5	1,475	Very slightly affected
B. pullorum 31	5	1,650	Very slightly affected

In these experiments control animals have been inoculated in each case. The filtrates were tested for sterility before being injected, and blood from the heart and liver of the animals that died was tested also

for sterility. It appears that mice, guinea-pigs, and chickens are quite resistant toward the toxin. Rabbits, however, are more susceptible. The fatal dose when injected intraperitoneally is about 2 c c of a 5-day bouillon filtrate for rabbits weighing about 2,000 gm. There is some variation in the amount of toxin produced by different strains. The symptoms appear from 1-2 hours after inoculation. There may be no special symptoms or there may be respiratory and intestinal disturbances. The respiratory symptoms are manifested by marked dyspnea; the intestinal disturbances, by the discharge of many fecal balls or even liquid feces and large amounts of urine. Death may be preceded by a violent struggle, or the animal may gradually expire. Death usually occurs within 24 hours. Necropsy shows often a congested liver and spleen. Hemorrhages into the thymus have been observed in a large percentage of the cases. The heart is dilated and there may be hemorrhages under the pericardium or endocardium. Microscopic examination of the tissues shows congestion of liver, kidneys and spleen. It appears that the toxin formed by *B. pullorum* or *B. sanguinarium* produces the same effect when injected into laboratory animals. The strength of the toxin formed is approximately the same for the two organisms, but there is some variation with different strains of both organisms.

SUMMARY AND CONCLUSIONS

There is little exact knowledge at present concerning the distribution of *B. pullorum* and *B. sanguinarium*, but it appears that both cause considerable losses to poultry raisers in many parts of the United States. There has apparently been some confusion at times in differentiating *B. avisepticus* from *B. sanguinarium*. *B. avisepticus*, however, may generally be distinguished from *B. sanguinarium* by its action in milk, indol production, fermentation of carbohydrates, agglutination reaction and pathogenesis. *B. pullorum* and *B. sanguinarium* do not produce indol, generally form hydrogen sulphid in a lead acetate medium, and produce a temporary acidity in milk, but later alkali is formed. *B. sanguinarium* usually produces alkali in a shorter time than *B. pullorum*. The casein is digested by most strains of these organisms. As regards fermentation, *B. pullorum* produces acid and generally gas in several carbohydrates. *B. sanguinarium* produces acid but no gas in the same carbohydrates, and in addition produces acid in dulcitol and maltose. There is some variation in the

reactions in maltose. Some strains of *B. pullorum* produce slight amounts of acid after several days' incubation and two strains have produced acid and gas quite promptly in maltose.

Agglutination tests with these organisms have shown that there is an antigenic relation between these organisms and *B. typhosus*, *B. enteritidis*, and *B. abortus-equinus*. Such antigenic relations were not observed between these avian strains and *B. avisepticus*, *B. dysenteriae*, *B. paratyphosus* A and B, *B. suipestifer*, *B. proteus*, and *B. coli*. Absorption tests will differentiate quite readily between *B. typhosus* and these avian strains. Agglutination and absorption tests with serums of rabbits immunized toward these avian types suggest an antigenic relation between *B. typhosus*, *B. enteritidis*, and these avian strains.

Feeding experiments indicate that laboratory cultures of these organisms will rarely produce an injurious effect on laboratory animals. Two freshly isolated strains of *B. sanguinarium* and one of *B. pullorum* were also fed to various animals without producing serious effects. It has been observed from inoculations that the rabbit is the most susceptible laboratory animal to the strains studied. It generally requires from 2-3 c.c. of a 24-hour broth culture injected intraperitoneally to produce a fatal infection in a rabbit weighing about 2,000 gm. A few freshly isolated strains were not more virulent for rabbits, guinea-pigs, mice and rats than cultures kept in the laboratory for some time. *B. pullorum* and *B. sanguinarium* produce a toxin when grown under proper conditions which is quite poisonous to rabbits. The action of the toxin produced appears to be the same for the two strains.

These avian strains may be differentiated from *B. typhosus* by their lack of motility, their fermentation reactions in rhamnose and sorbite, and absorption tests with immune serums. Serologic tests and certain fermentative reactions may be used to differentiate these organisms from *B. dysenteriae*. They may be differentiated from other members of the paratyphoid-enteritidis group by morphologic and cultural characteristics, and serologic tests. *B. avisepticus*, as has been mentioned before, may be differentiated in many ways from *B. sanguinarium* and *B. pullorum*.

B. pullorum may be distinguished from *B. sanguinarium* by the inability of the former to ferment dulcitol, while the latter ferments

this carbohydrate. Also, the former organism does not generally produce acid in maltose and generally produces gas in several of the carbohydrates. *B. sanguinarium*, on the other hand, generally produces acid promptly in maltose and does not produce gas in any of the carbohydrates. Rhamnose is fermented promptly by *B. pullorum*, while *B. sanguinarium* does not produce acid before 48 hours' incubation. It appears, therefore, that there are sufficient differences between *B. sanguinarium* and *B. pullorum* to regard them as separate types.

